Section 12 - Summary Progress Report

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1. Experiments Designed to Enhance Protective Anti-Tumor Immune Responses.

a) BCG in mice

Studying a neuroblastoma which arose spontaneously in the A mouse strain, experiments were carried out to investigate the effect of live BCG on the rejection of this tumor in the syngeneic host. This neuroblastoma is uniformly lethal for adult A strain mice in a dose of 10^2 tumor cells in about 15 days. Injection of live BCG with the tumor inoculum protected the mice against a tumor inoculum 100 times larger (10^4 cells). To explore the mechanism of this effect and more precisely whether BCG administered with the tumor was able to increase specific tumor immunity, the mice which survived the 10^4 tumor cell challenge and BCG were reinoculated with 5×10^3 neuroblastoma cells at the same time as control A strain mice. A very significant increase in survival time following this inoculum was observed in the BCG treated group compared to the control group. These findings indicate some increase in tumor immunity as a consequence of BCG administration with tumor cells in mice.

b) The allogeneic effect caused by graft versus host reactions.

After the experimental conditions required for the injection of allogeneic immunocompetent cells to exercise adjuvant effects had been ascertained in mice and rats, experiments have been initiated to test the protective action of the allogeneic effect on the rejection of solid syngeneic tumors in these two species. The following tumors have been studied in mice:

Mammary carcinomas in C_3H and DBA_2 strains

Rhabdomyosarcomas in DBA_2 strain

Fibrosarcoma in DBH_1 strain

In rats two tumors are being investigated, a mammary adenocarcinoma induced by nickel iodide in the Fisher 344 strain and a glioblastoma, which arose spontaneously in the Wistar-Furth strain. GVH reactions are induced by the injection of appropriate numbers of allogeneic immunocompetent cells. So far in preliminary experiments,

GVH reactions, or the allogeneic effects, were successful in decreasing very significantly the growth of transplanted glioblastoma in its syngeneic host. Further experiments with all the above mentioned tumors are in progress.

2. Purification of Hodgkin's Disease Tumor Associated Antigens. (Proc. Nat. Acad. of Sciences (In press), preprint attached.

These studies designed to characterize the antigens associated with Hodgkin's Disease were carried out in collaboration with Dr. Order of the Department of Radiation Therapy.

Two antigens which exist in high frequency in tumor tissues of patients with Hodgkin's Disease have been obtained in relatively concentrated form. Extracts of Hodgkin's spleen tumor tissue when subjected to chromatography on Sephadex G-200 separate into three major protein peaks of which only the first (peak I) possesses the predominant antigenic activities associated with the disease. Antigenic analysis performed with hyperimmune rabbit antisera obtained after repeated immunizations with peak I proteins demonstrated that this fraction contained both F and S antigens associated with Hodgkin's Disease and small contaminant amounts of an antigen associated with normal lymphocytes. The tissue distribution patterns of the Hodgkin's Disease tumor-associated antigens suggest that they both originate in lymphoid tissues and that F antigen may represent a product of reactive lymphocytes while S antigen may be a dedifferentiation antigen expressed in very immature lymphocytes.

3. Studies on the Mechanism of Immune Cytolysis by Sensitized Thymus Derived Cells.
Inhibition of immune cell-mediated killing by Heparin.
(to be submitted to the J Exp Med) preprint attached.

Heparin retarded the cell-mediated lysis of mouse ascitic tumor cells or normal spleen cells by effector spleen cells from allo-immunized mice. The retardation was immediately reversible by removal of the heparin. Heparin reduced the rate of cell lysis without preventing the eventual complete destruction of the target cell population. The completion of killing was not due to loss of heparin activity or desensitization of the killer cells to heparin. At a given concentration of heparin, the amount of inhibition depended on the target cell type employed, but was relatively independent of the speed with which lysis progressed. Retardation was independent of divalent cation concentration and serum concentration, and was not affected by depletion of red blood cells or adherent cells. Heparin did not inhibit incorporation of tritiated thymidine in mixed lymphocyte cultures, and hence was not non-specifically toxic for lymphocytes. The dose responses of killing to three other highly sulfated polymers were similar to that for heparin. This indicates that the inhibition is due to the high charge density and polyanionic character of heparin, and not to an impurity. The anti-coagulant activity of these polyanions was not correlated with their inhibitory activity for cell-mediated killing.

When heparin is added to cultures after about 10% specific release of chromium has occurred, it has virtually no effect on subsequent killing. This is not due to desensitization of the killer cells to heparin as a result of the previous killing experience. Hence, there is an early heparin-sensitive killer-target interaction which, once it has occurred, cannot be reversed by heparin. This sensitive interaction seems likely to be the formation of a close contact between the killer and target cells.